CD27 is a new promising T-cell co-stimulatory target for cancer immunotherapy

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Society for Immunotherapy of Cancer

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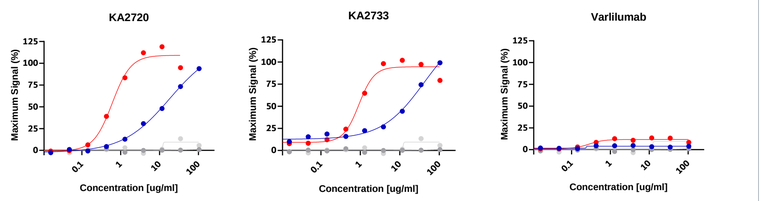
Introduction

- CD27, a member of the TNFRS Family, promotes T cell co-activation, proliferation, clonal expansion, and differentiation into antigen-specific cytotoxic and memory T cells after stimulation with its ligand CD70
- The costimulatory signal of CD27 on T cells is mediated via the NFκB pathway as well as the phosphatidylinositol 3 kinase and the protein kinase B pathways
- CD27 signaling influences the innate immune response via direct activation of NK cells and subsequent secretion of pro-inflammatory cytokines
- CD27 plays a central role in immunological responses, and by promoting T cell and NK cell activation, it contributes to anti-tumor immunity

Objectives

- Perform *in vitro* and *in vivo* assessments to identify an anti-CD27 agonist antibody lead candidate

Anti-CD27 antibodies induce NFκB-mediated CD27 signaling in both crosslinked and soluble formats



Antibody	Soluble format EC50 (ug/mL)	Soluble format EC50 (nM)	Cross-linking format EC50 (ug/mL)	Cross-linking format EC50 (nM)
KA2733	18	123	0.9	6.1
KA2720	33	220	1.3	8.7
Varlilumab	>104	>693	>104	>693
hIgG1	>104	>693	>104	>693

Figure 1. NFκB-Luc2/CD27 Jurkat cells (Promega) were seeded at 2e5 cells/well in the 96-well plates and incubated with anti-CD27 antibodies. Goat anti-human IgG F(ab')2 cross-linking antibodies were added at a 1:2 ratio and incubated for 4 hours at 37^{0}C. NFκB reporter induction was measured by luminescence in the presence of Bio-Glo reagent (Promega).

Anti-CD27 antibodies and CD70 bind to distinct epitopes on CD27

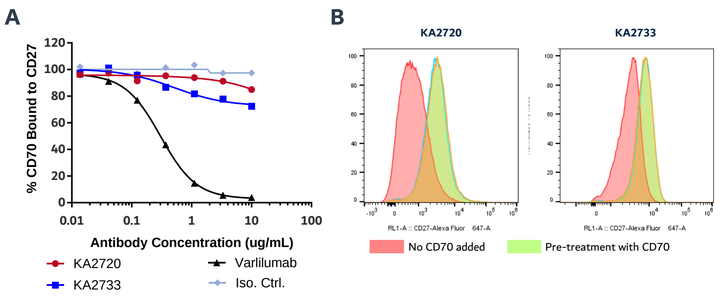


Figure 2: (A) hCD70 competition ELISA was used to measure the binding of soluble human CD70-ECD-mFc to immobilized human CD27-ECD-rFc in the presence of anti-CD27 antibodies. Bound CD70 was detected using HRP anti-mouse IgG (H+L).
(B) hCD70 competition cell binding assay using CD27$^+$ Raji cells: cells were stained with anti-CD27 mAbs-AF-647 for 60 min without hCD70-ECD trimer pre-treatment (red) or with hCD70-ECD trimer 30 min pre-treatment (green), fixed and analyzed via flow cytometry (Attune NxT).

Results

Anti-CD27 antibodies mediate NFκB activation in synergy with CD70

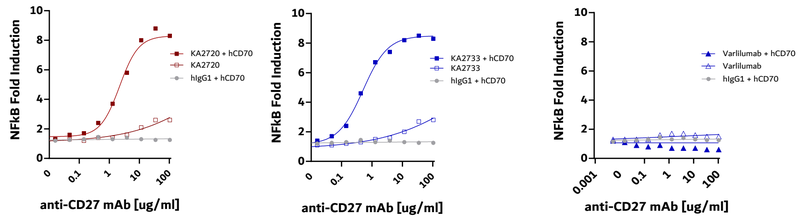
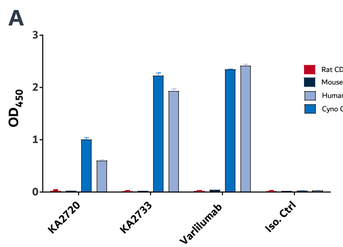


Figure 3. NFκB-Luc2/CD27 Jurkat cells (Promega) were seeded at 2e5 cells/well in the 96-well plates and incubated with anti-CD27 antibodies and 0.1 ug/mL hCD70-ECD trimer (R&D) for 4 hrs at 37^{0}C. NFκB reporter induction was measured by luminescence in the presence of Bio-Glo reagent (Promega).

Anti-CD27 antibodies bind specifically to human and cynomolgus monkey CD27



B

	Human CD27	Cynomolgus CD27	Mouse CD27
Antibody	KD (nM)	KD (nM)	KD (nM)
KA2733	1.5	2.3	BLQ
KA2720	7.7	11	BLQ
Varlilumab	<0.001	<0.001	BLQ
Iso. Ctrl	BLQ	BLQ	BLQ

Figure 4. Anti-CD27 antibodies bind to human and cynomolgus monkey CD27 similarly but not to the rat and mouse CD27 (**A**) Binding of anti-CD27 antibodies to immobilized human CD27, cynomolgus monkey CD27, rat CD27 or mouse CD27 measured by **ELISA**. Error bars represent SD (standard deviation). (**B**) **Kinetics sensorgrams** of anti-CD27 antibodies on immobilized human, cynomolgus monkey, or mouse CD27 generated using **Octet** (FortéBio, Sartorius AG). Kinetic studies were performed using CD27-ECD-His-tag loaded onto HIS1K dip and read biosensors. A 1:1 global curve fitting analysis was performed to determine equilibrium (KD), association (ka), and dissociation (kdis) rate constants. BLQ stands for below the limit of quantification.

Anti-CD27 antibodies show high specificity

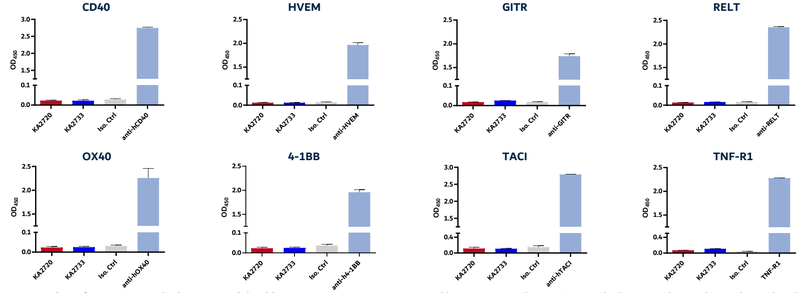


Figure 5. Binding of anti-CD27 antibodies to immobilized human TNFRSF proteins measured by ELISA. Bound anti-CD27 antibodies were detected using biotinylated anti-human IgG (light chain) followed by streptavidin HRP. Error bars represent SD (standard deviation).

Anti-CD27 agonistic antibodies exhibit additive antitumor efficacy with other immune checkpoint inhibitors

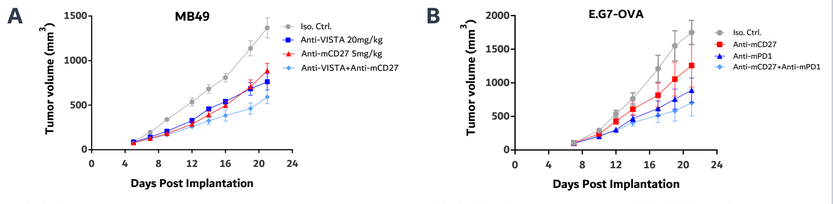


Figure 6. (A) Plot of mean tumor volumes vs. time following subcutaneous implantation of 0.5x10^6 MB49 cells/mouse into female hVISTA-KI C57BL/6 homozygous mice. Five days after cell implantation, mice received i.p. injection of 20mg/kg of isotype control antibody (Iso. Ctrl.), 5mg/kg of anti-mCD27 agonistic antibody, or 20mg/kg of anti-VISTA mAb alone or in combination two times a week for 3 weeks. **(B)** Plot of mean tumor volumes vs. time following subcutaneous implantation of 1x10^6 EG7-OVA cells/mouse into female C57BL/6J mice. Seven days after cell implantation, mice received i.p. injection of either 10mg/kg of isotype control antibody (Iso. Ctrl.), 5mg/kg of anti-mPD1, 10mg/kg of anti-mCD27 agonistic antibody alone or in combination two times per week for 3 weeks. Error bars represent SEM.

Anti-CD27 antibodies demonstrate *in vivo* anti-tumor efficacy as a monotherapy or in combination with anti-PD1 in different tumor models

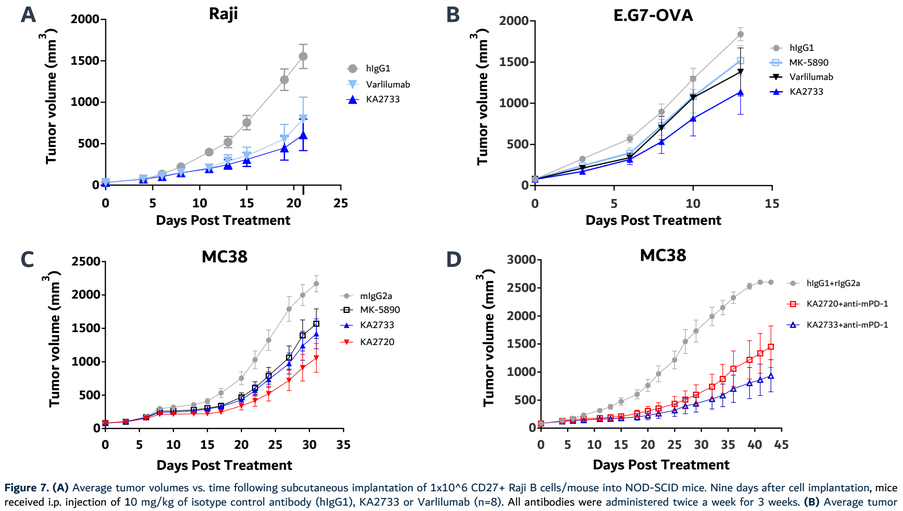


Figure 7. (A) Average tumor volumes vs. time following subcutaneous implantation of 1x10^6 CD27+ Raji B cells/mouse into NOD-SCID mice. Nine days after cell implantation, mice received i.p. injection of 10 mg/kg of isotype control antibody (hIgG1), KA2733 or Varlilumab (n=8). All antibodies were administered twice a week for 3 weeks. **(B)** Average tumor volumes vs. time following subcutaneous implantation of 1x10^6 E.G7-OVA cells/mouse into hCD27KI C57BL/6 mice. Nine days after cell implantation, mice received i.p. injection of 10 mg/kg of isotype control antibody (hIgG1), KA2733, MK-5890 or Varlilumab (n=8). All antibodies were administered twice a week for 3 weeks. **(C)** Average tumor volumes vs. time following subcutaneous implantation of 1x10^6 MC38 cells/mouse into hCD27-KI C57BL/6 mice. Seven days after cell implantation, mice received i.p. injection of 5 mg/kg of isotype control antibody (mIgG2a), KA2720, KA2733 or MK-5890 on a mouse IgG2a backbone (n=10). All antibodies were administered twice a week for 4 weeks. **(D)** Average tumor volumes vs. time following subcutaneous implantation of 1x10^6 MC38 cells/mouse into hCD27-KI C57BL/6 mice. Seven days after cell implantation, mice received i.p. injection of 5 mg/kg of each isotype control antibody (hIgG1 and rIgG2a), and 5mg/kg of mPD1 combined with 5mg/kg of KA2720 or KA2733 (n=10). All antibodies were administered twice a week for 4 weeks. Error bars represent SEM.

Anti-CD27 antibodies activate T cells and NK cells

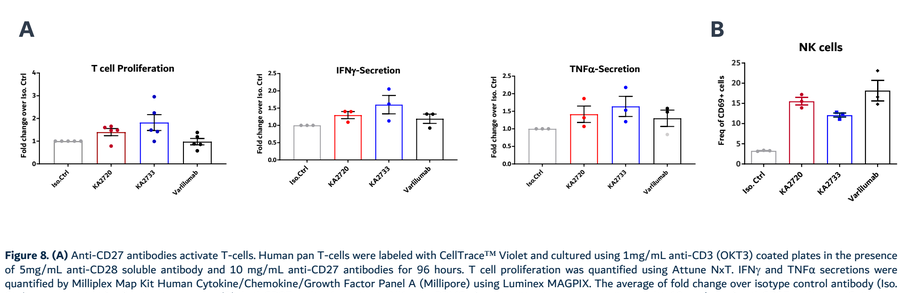


Figure 8. (A) Anti-CD27 antibodies activate T-cells. Human pan T-cells were labeled with CellTrace™ Violet and cultured using 1mg/mL anti-CD3 (OKT3) coated plates in the presence of 5mg/mL anti-CD28 soluble antibody and 10 mg/mL anti-CD27 antibodies for 96 hours. T cell proliferation was quantified using Attune NxT. IFNγ and TNFα secretions were quantified by Milliplex Map Kit Human Cytokine/Chemokine/Growth Factor Panel A (Millipore) using Luminex MAGPIX. The average of fold change over isotype control antibody (Iso. Ctrl) was calculated for five healthy donors. **(B)** Anti-CD27 antibodies activate NK cells. PBMCs from healthy donors were cultured with 10 mg/mL of anti-CD27 antibodies. Cells were harvested after 96 hours, stained with live dead dye and surface markers for NK cells, fixed with BD Cytofix buffer, and analyzed using Attune Nxt Flow Cytometry. CD56dim CD16bright cells were evaluated for activation marker CD69. The frequency of CD69+ cells in CD56dim CD16bright cells is shown. Error bars represent SEM.

Conclusions

Kineta's selected lead anti-CD27 agonist mAb:

- Demonstrates high affinity and specificity to both human and cynomolgus monkey CD27 but not to rodent CD27
- Shares a different binding site from CD70 on CD27
- Induces strong NFκB-mediated signaling in a soluble or cross-linked format
- Mediates NFκB activation in synergy with CD70
- Drives strong T cell activation and proliferation as well as NK cell activation
- Demonstrates *in vivo* antitumor efficacy as a single agent or in combination with other immunotherapies in solid and hematological tumor models